

What is claimed is:

- 5 1. An electrochemical method for detecting a target polynucleotide, comprising:  
obtaining a first electrochemical signal comprising a portion arising from a  
first amount of a probe molecule not intercalated with the target polynucleotide;  
modifying an amount of target polynucleotide in fluid communication with  
the first amount of probe molecule; and  
10 obtaining a second electrochemical signal comprising a portion arising from  
a second amount of probe molecule not intercalated with the target polynucleotide.
2. The electrochemical method of claim 1, wherein, upon modifying an amount of  
target polynucleotide, a portion  $\Delta$  of the first amount of probe molecule intercalates with the  
target polynucleotide, the second electrochemical signal being different from the first  
15 electrochemical signal by an amount indicative of  $\Delta$ .
3. The electrochemical method of claim 1, wherein the first and second  
electrochemical signals are substantially free of an electrochemical signal from intercalated  
probe molecule.
4. The electrochemical method of claim 1, wherein probe molecule is substantially  
20 free of polynucleotides having a length of at least 8 bases.
5. The electrochemical method of claim 4, wherein the first and second  
electrochemical signals arise from an electrochemically active moiety of the probe molecule  
that is free of purines.
6. The electrochemical method of claim 5, wherein the probe molecule comprises  
25 at least two cyclic groups.
7. The electrochemical method of claim 5, wherein the probe molecule comprises  
at least three 6 membered rings.
8. The electrochemical method of claim 6, wherein the probe molecule comprises  
an anthracycline, methylene blue, or derivative thereof.
- 30 9. The electrochemical method of claim 5, wherein the probe molecule is selected  
from the group consisting of daunomycin, doxorubicin, methylene blue, toluidine blue O,  
azure A, azure B, azure C, and thionin.<sup>†</sup>

10. The electrochemical method of claim 5, wherein the probe molecule comprises (a) a polynucleotide having a sequence sufficiently complementary to a sequence of the target polynucleotide to form a duplex therewith and (b) an electrochemically active moiety that is free of purines, the first and second electrochemical signals arising from the electrochemically active moiety.

11. The electrochemical method of claim 10, further comprising detecting a second polynucleotide, the method comprising:

obtaining a third electrochemical signal comprising a portion arising from a first amount of a second probe molecule not intercalated with the target polynucleotide or the second target polynucleotide, the second probe molecule comprising (a) a polynucleotide having a sequence sufficiently complementary to a sequence of the second target polynucleotide to form a duplex therewith and different from the sequence of the polynucleotide of the probe molecule and (b) an electrochemically active moiety that is free of purines;

modifying an amount of the second target polynucleotide in fluid communication with the first amount of the second probe molecule; and

obtaining a second electrochemical signal comprising a portion arising from a second amount of the second probe molecule not intercalated with either the target polynucleotide or the second polynucleotide.

12. The electrochemical method of claim 1, wherein the first and second electrochemical signals are obtained using an electrode and the second electrochemical signal is obtained without contacting the electrode with fresh probe molecule intermediate obtaining the first and second electrochemical signals.

13. The electrochemical method of claim 1, wherein the second electrochemical signal is substantially free of a portion arising from an oxidation or reduction of guanine residues, if present, of the target polynucleotide.

14. An electrochemical method for detecting a target polynucleotide, comprising: obtaining a first electrochemical signal from a first amount of a probe molecule, the probe molecule having a first electrochemical activity when not intercalated with the target polynucleotide and a second, different electrochemical activity when intercalated with the target polynucleotide;

modifying an amount of target polynucleotide in fluid communication with the first amount of probe molecule whereupon a portion  $\Delta$  of the first amount of probe molecule intercalates with the target polynucleotide; and

obtaining a second electrochemical signal from a second amount of probe molecule.

15. The electrochemical method of claim 14, wherein the second electrochemical activity is lower than the first electrochemical response.

5 16. The electrochemical method of claim 14, wherein the second electrochemical signal is different from the first electrochemical signal by an amount indicative of  $\Delta$ .

17. The electrochemical method of claim 14, wherein the probe molecule is substantially free of polynucleotides having a length of at least 8 bases.

18. The electrochemical method of claim 14, wherein the probe molecule is selected  
10 from the group consisting of daunomycin, doxorubicin, methylene blue, toluidine blue O, azure A, azure B, azure C, and thionin.

19. The electrochemical method of claim 18, wherein the probe molecule comprises  
15 (a) a polynucleotide having a sequence sufficiently complementary to a sequence of the target polynucleotide to form a duplex therewith and (b) an electrochemically active moiety that is free of purines, the first and second electrochemical signals arising from the electrochemically active moiety.

20. The electrochemical method of claim 14, wherein the first and second electrochemical signals are obtained using an electrode and the second electrochemical signal is obtained without contacting the electrode with fresh probe molecule intermediate  
20 obtaining the first and second electrochemical signals.

21. An electrochemical method for detecting a target polynucleotide, comprising:  
obtaining a first electrochemical signal from a first amount of a probe molecule immobilized with respect to an electrode;

25 modifying an amount of target polynucleotide in fluid communication with the first amount of probe molecule; and

obtaining a second electrochemical signal from a second amount of the probe molecule immobilized with respect to the electrode.

22. The electrochemical method of claim 21, wherein the probe molecule is substantially free of polynucleotides having a length of at least 8 bases.

30 23. The electrochemical method of claim 22, wherein the first and second electrochemical signals arise from an electrochemically active moiety of the probe molecule that is free of purines.

24. The electrochemical method of claim 21, wherein the second electrochemical signal is substantially free of a portion arising from an oxidation or reduction of guanine residues, if present, of the target polynucleotide.
25. The electrochemical method of claim 21, wherein the probe molecule is selected from the group consisting of daunomycin, doxorubicin, methylene blue, toluidine blue O, azure A, azure B, azure C, and thionin.
26. The electrochemical method of claim 21, wherein the first and second electrochemical signals are obtained using an electrode and the second electrochemical signal is obtained without contacting the electrode with fresh probe molecule intermediate obtaining the first and second electrochemical signals.
27. An electrochemical method for detecting a target polynucleotide, comprising:  
using an electrode to obtain a first electrochemical signal, the first electrochemical signal indicative of a first amount of probe molecule and arising from a polynucleotide-free electrochemically active moiety of the probe molecule; and  
using the electrode to obtain a second electrochemical signal, the second electrochemical signal indicative of a second amount of probe molecule and arising from the polynucleotide-free electrochemically active moiety of the probe molecule.
28. The electrochemical method of claim 27, further comprising, intermediate obtaining the first and second electrochemical signals, modifying an amount of target polynucleotide in fluid communication with the electrode; and
29. The electrochemical method of claim 27, comprising determining the presence of the target polynucleotide based upon the first and second electrochemical signals.
30. The electrochemical method of claim 27, comprising:  
prior to using the electrode to obtain the first electrochemical signal and with the electrode (a) dry and (b) having an amount of probe molecule associated therewith, contacting the electrode with a liquid.
31. The electrochemical method of claim 30, wherein, prior to contacting the electrode with a liquid, the electrode is substantially free of the target polynucleotide.
32. The electrochemical method of claim 27, comprising:  
prior to using the electrode to obtain the second electrochemical signal and with the electrode (a) substantially free of the target polynucleotide and (b) having an amount of probe molecule associated therewith, contacting the electrode with a liquid comprising the target polynucleotide.

33. The electrochemical method of claim 32, wherein prior to contacting the electrode with the liquid, the electrode is substantially free of polynucleotides having sequences that are (a) at least 8 bases in length and (b) sufficiently complementary to a sequence of the target polynucleotide to form a duplex therewith.
- 5 34. The electrochemical method of claim 33, wherein prior to contacting the electrode with a liquid, the electrode is dry.
35. The electrochemical method of claim 27, wherein the probe molecule is substantially free of polynucleotides having a length of at least 8 bases.
36. The electrochemical method of claim 27, wherein the polynucleotide-free  
10 electrochemically active moiety of the probe molecule is free of purines.
37. The electrochemical method of claim 27, wherein the second amount of probe molecule is not intercalated with the target polynucleotide.
38. The electrochemical method of claim 37, wherein the first amount of probe molecule is not intercalated with the target polynucleotide.
- 15 39. The electrochemical method of claim 27, wherein, at the some time prior, the electrode is dry.
40. The electrochemical method of claim 28, wherein modifying the amount of target polynucleotide in fluid communication with the electrode comprises subjecting the target polynucleotide in fluid communication with the electrode to at least one amplification  
20 cycle.
41. The electrochemical method of claim 27, wherein the first and second electrochemical signals arise from the application of a potential difference between the electrode and a reference electrode, the potential difference being between -1.2 V and 1 V as against a Ag/AgCl reference electrode.
- 25 42. The electrochemical method of claim 28, wherein modifying the amount of target polynucleotide in fluid communication with the electrode comprises increasing the amount of target polynucleotide in fluid communication with the electrode and a magnitude of the second electrochemical signal is less than a magnitude of the first electrochemical signal.
- 30 43. The electrochemical method of claim 27, wherein a difference between the first and second amounts of target polynucleotide is less than  $5 \times 10^{-9}$  molar.

44. The electrochemical method of claim 28, wherein modifying the amount of polynucleotide in fluid communication with the electrode comprises increasing the amount of polynucleotide in fluid communication from zero to a non-zero amount.

45. The electrochemical method of claim 27, wherein the probe molecule is selected from the group consisting of daunomycin, doxorubicin, methylene blue, toluidine blue O, azure A, azure B, azure C, and thionin.

46. An electrochemical method for detecting a polynucleotide, comprising:  
obtaining a first electrochemical signal from a first amount of probe molecule in the presence of a first polynucleotide and a second polynucleotide, the first and second polynucleotides being sufficiently complementary to form a duplex;  
10 subjecting the first and second polynucleotides to at least one of an annealing step or a melting step in the presence of the first amount of probe molecule; and  
then, obtaining a second electrochemical signal from the probe molecule.

47. The electrochemical method of claim 46, wherein the first electrochemical signal is obtained at a temperature below the melting point of the duplex region and the second electrochemical signal is obtained at a temperature at least as great as the melting point of the duplex.

48. The electrochemical method of claim 46, wherein subjecting the first and second polynucleotides to at least one of an annealing step or a melting step further comprises  
20 subjecting the first and second polynucleotides to at least one amplification step intermediate obtaining the first and second electrochemical signals and in the presence of the probe molecule.

49. The electrochemical method of claim 46, wherein the first electrochemical signal is obtained using an electrode and the method comprises contacting the electrode with a liquid prior to obtaining the first electrochemical signal, the electrode being dry prior to  
25 being contacted with the liquid.

50. The electrochemical method of claim 46, wherein the first electrochemical signal is obtained using an electrode and the method comprises contacting the electrode with a liquid prior to obtaining the first electrochemical signal, prior to the contacting step, the electrode comprising at least a portion of the first amount of probe molecule reversibly  
30 immobilized with respect thereto.

51. The electrochemical method of claim 46, wherein the probe molecule is substantially free of polynucleotides having a length of at least 8 bases.

52. The electrochemical method of claim 51, wherein the first and second electrochemical signals arise from an electrochemically active moiety of the probe molecule that is free of purines.

53. The electrochemical method of claim 51, wherein the first and second  
5 electrochemical signals are substantially free of a contribution arising from an oxidation or a reduction of guanine.

54. A method for preparing a device for electrochemically determining the presence of a polynucleotide, comprising:

10 contacting an electrode with a liquid comprising an intercalating compound, the intercalating compound having a first electrochemical activity when not intercalated with a double stranded polynucleotide and a second, different electrochemical activity when intercalated with a double stranded polynucleotide; and  
drying the electrode.

55. The method of claim 54, wherein the probe molecule is free of purines.

15 56. The electrochemical method of claim 54, wherein the probe molecule is selected from the group consisting of daunomycin, doxorubicin, methylene blue, toluidine blue O, azure A, azure B, azure C, and thionin.

57. The method of claim 54, further comprising:

20 preparing the electrode by a method comprising:  
preparing a flowable mixture comprising a plurality of conductive particles and the probe molecule.

58. The method of claim 54, wherein, prior to drying the electrode, the method excludes binding polynucleotide sequences having a length of greater than 8 bases to the electrode.

25 59. The method of claim 49, further comprising:  
preparing a microfluidic device comprising the electrode.

60. The method of claim 59, comprising, after drying the electrode, sealing the microfluidic device in a hermetic enclosure.

30 61. A method for preparing a device for electrochemically determining the presence of a polynucleotide, comprising:

contacting an electrode with a liquid comprising an intercalating compound, the intercalating compound having a first electrochemical activity when not intercalated

with a double stranded polynucleotide and a second, different electrochemical activity when intercalated with a double stranded polynucleotide; and  
sealing the microfluidic device in an enclosure.

62. The method of claim 61, wherein the probe molecule is free of purines.

5 63. The method of claim 61, wherein, prior to sealing the microfluidic device, the method excludes binding polynucleotide sequences having a length of greater than 8 bases to the electrode.

64. The method of claim 61, wherein the probe molecule is selected from the group consisting of daunomycin, doxorubicin, methylene blue, toluidine blue O, azure A, azure B,  
10 azure C, and thionin.

65. The method of claim 61, further comprising:  
preparing the electrode by a method comprising:  
preparing a flowable mixture comprising a plurality of conductive  
particles and the probe molecule.

15